UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

FABRÍCIA CARDOSO

# *In situ* effect of a proanthocyanidin mouthrinse on dentin submitted to erosion

# Efeito *in situ* de uma solução de proantocianidina sobre a dentina submetida à erosão dentária

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Orientador: Prof. Dr. Heitor Marques Honório

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# DEDICATÓRIA

## DEDICATÓRIA

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## AGRADECIMENTOS

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*E tudo o que pedires em oração, crendo, o recebereis."*

*Mateus 22:21*

# ABSTRACT

# ABSTRACT

***In situ* effect of a proanthocyanidin mouthrinse on dentin submitted to erosion**

The organic matrix is of great importance in the process of loss of dental tissue because it functions as a barrier that prevents the diffusion of the acids to the tissues. For its degradation to be avoided, some agents have been tested in an attempt to inhibit the MMPs, the enzymes responsible for this process. Proanthocyanidin has been shown to be efficient in inhibiting these enzymes and therefore the aim of this study *in situ* was to evaluate the protective effect of a mouthrinse based on proanthocyanidin applied on the dentin submitted to erosion. Seven volunteers wore 2 palatal devices in each phase. The groups under study were: First Phase/ G1 - Erosive challenge with acid drink (Coca-cola®) before dentin treatement with 10% proanthocyanidin mouthrinse (neutralized to pH 7.0, Experimental group 1), G2 - Erosive challenge with acid drink (Coca-cola®) before dentin treatement with 10% proanthocyanidin mouthrinse (without neutralization, pH 3.0, Experimental group 2). Second Phase/ G3 - Erosive challenge with acid drink (Coca-cola®) before dentin treatement with 0.12% Chlorhexidine mouthrinse (pH 7.0, Positive control group), G4 - Erosive challenge with acid drink (Coca-cola®) with no previous treatment (Negative control group). Treatments with different mouthrinses were applied once after the second erosive challenge, for 5 minutes. Volunteers continuously wore the oral appliance and for the erosive cycling, each device was immersed into the 32 ml of acid beverage, 3 times a day for 5 minutes during 5 days. Profilometry was used to quantify the dentin loss (µm). Data were analyzed by Repeated measures ANOVA followed by LSD Fishers's test ($p<0.05$). G1 ($1.17^{a} \pm 0.69$) and G3 ($1.22^{a} \pm 0.25$) showed significant lower wear values with no statistical difference between them. There was also no significant differences between G2 ($2.99^{b} \pm 1.15$) and G4 ($2.29^{b} \pm 1.13$) resulting in more wear when compared to others groups. This study suggest that 10% neutralized proanthocyanidin mouthrinse could be a good strategy to diminish dentin wear progression.



**Keywords:** Proanthocyanidin; Tooth wear; Dentin; Erosion.

# RESUMO

# RESUMO

**Efeito *in situ* de uma solução de proantocianidina sobre a dentina submetida à erosão dentária**

A matriz orgânica é de grande importância no processo de perda de tecido dental porque funciona como uma barreira que impede a difusão dos ácidos para os tecidos. Para evitar a sua degradação, alguns agentes foram testados numa tentativa de inibir as MMPs, enzimas responsáveis por este processo. A proantocianidina mostrou ser eficiente na inibição das mesmas e, portanto, o objetivo deste estudo *in situ* foi avaliar o efeito protetor de um enxaguatório com base em proantocianidina aplicada na dentina submetida à erosão. Este estudo cruzado simples-cego foi realizado em 2 fases de 5 dias cada. Sete voluntários usaram 2 dispositivos palatinos em cada fase. Os grupos estudados foram: Primeira Fase / G1 - Desafio Erosivo com bebida ácida (Coca-cola®) antes do tratamento da dentina com enxaguatório bucal de proantocianidina 10% (pH neutralizado a 7,0, Grupo Experimental 1), G2 - Desafio Erosivo com bebida ácida -cola®) antes do tratamento na dentina com enxaguatório bucal de proantocianidina a 10% (sem neutralização, pH 3,0, grupo experimental 2). Segunda Fase / G3 - Desafio erosivo com bebida ácida (Coca-cola®) antes do tratamento na dentina com 0,12% de enxaguatório bucal de Clorhexidina (pH 7,0, controle positivo), G4 - Desafio erosivo com bebida ácida (Coca-cola®) sem tratamento (controle negativo). Os tratamentos com diferentes enxaguatórios foram aplicados uma vez após o segundo desafio erosivo, durante 5 minutos. Os voluntários continuamente usavam o aparelho oral e para o ciclo erosivo, cada dispositivo foi imerso em 150 ml de bebida ácida, 3 vezes ao dia durante 5 minutos durante 5 dias. A Perfilometria foi utilizada para quantificar a perda de dentina (µm). Os dados foram analisados por ANOVA de medidas repetidas seguido por teste de Fishers LSD ($p < 0,05$). G1 ($1,17^{a} \pm 0,69$) e G3 ($1,22^{a} \pm 0,25$) mostraram valores de desgaste significativamente menores, sem diferença estatística entre eles. Também não houve diferenças significativas entre G2 ($2,99^{b} \pm 1.15$) e G4 ($2,29^{b} \pm 1.13$), resultando em maior desgaste quando comparado a outros grupos. Este estudo sugere que o enxaguatório bucal de proantocianidina 10% neutralizada poderia ser uma boa estratégia para diminuir a progressão do desgaste dentinário.



**Palavras-chave**: Proantocianidina. Desgaste dentário. Dentina. Erosão.

# TABLE OF CONTENTS

1 **INTRODUCTION** ..... 17

2 **DISCUSSION** ..... 24

3 **CONCLUSION OR FINAL CONSIDERATIONS** ..... 29

**REFERENCES** ..... 33

# 1 INTRODUCTION

# 1 INTRODUCTION

Dental caries, which for some decades was the major concern of dentists, have shown a decrease in incidence in developed and underdeveloped countries. Therefore, non-carious lesions have become more frequent and among them, tooth wear (MAHONEY, KILPATRICK, 2003; HUYSMANS, CHEW, ELLWOOD, 2011; AL-ZAREA, 2012; MOAZZEZ, BARTLETT, 2014).

The tooth wear is subdivided into three categories: attrition, abrasion and erosion (BARTLETT, 2006; GANSS, 2006; HARA, LUSSI, ZERO, 2006; WEST, JOINER, 2014). Attrition is the superficial loss of dental structure due to dental contacts involving occlusal and incisal surfaces during swallowing, phonation and in cases of bruxism . Abrasion is caused by mechanical forces, but not related to the occlusion, among which we can highlight the technique of toothbrushing the type of brush used and the presence of abrasive in the toothpaste (ECCLES, 1982; IMFELD, 1996; MAGALHÃES et al, 2009a, RIOS et al, 2006; RIOS et al, 2007; WEST, JOINER, 2014).

Erosion presents a more complex pathophysiology, characterized as chronic and chemical erosion caused by acids without the involvement of microorganisms. It may occur due to intrinsic or extrinsic mechanisms (LUSSI, JAEGGI, ZERO, 2004; KAIDONIS, 2012; CARVALHO, 2014; LUSSI, GANSS, 2014).

Intrinsic erosion is resulted from the action of hydrochloric acid present in the stomach, which is found in the oral cavity through vomiting (anorexia and bulimia), gastric content regurgitation, recurrent reflux or gastric dysfunction (SCHEUTZEL, 1996; ZERO, 1996; LUSSI, JAEGGI, ZERO, 2004; AMAECHI, HIGHAM, 2005; BARBOSA et al, 2009; UHLEN et al, 2014). Extrinsic erosion occurs due to excessive consumption of beverages and foods with a high level of acidity or environmental exposure to acidic agents (ZERO, 1996; CHEHAL et al, 2009; ISAKSSON et al, 2014; JAEGGI, LUSSI, 2006).

Studies have shown that the evolution of the disease brings to the patient discomforts such as pain and hypersensitivity (O'SULLIVAN et al, 1998; RYTOMAA et al, 1998; RIOS et al, 2007). Because of that, it is of paramount importance that preventive methods be employed in order to minimize such symptoms, since the more advanced stage of

the disease demands more time and greater cost to the patient (SERRA, MESSIAS, TURSSI, 2009).

For the curative and preventive measures to be effective, it is necessary to know the different composition of the structures involved in the disease process, in this case enamel and dentin, as well as its behavior in the evolution of this type of lesion.

Approximately 85% of dental enamel is composed of mineral, in the form of highly substitutable hydroxyapatite crystals arranged in prisms, the remainder composed by proteins and water. In situations where the pH is mainly below 4.5 these crystals are easily solubilized by the acids, making the dental surface softened, which results in the loss of its structural component, characterizing tooth wear (BUZALAF et al, 2010, HONÓRIO et al, 2008; RIOS et al, 2008; HONÓRIO et al, 2010; LUSSI A, GANSS, 2014; SIERPINSKA et al, 2013).

The dentin is composed of inorganic, organic material and water. The inorganic matrix is represented by replaceable hidroxyapatite crystals, poor in calcium, phosphate, but rich in magnesium and carbonate when compared to enamel hydroxyapatite (SILVERSTONE, HICKS, 1985). These crystals, located between and on collagen fibrils, are also smaller in size when compared to those found in dental enamel, allowing them a larger surface area which makes them more vulnerable to acid attack (SILVERSTONE, HICKS, 1985).

The organic matrix is composed of 90% collagen fibrils (type I collagen) and the remaining 10% is composed of dentin, proteoglycan and glycosaminoglycan phosphoproteins (SILVERSTONE, HICKS, 1985; PASHLEY et al, 2004; MAGALHÃES et al, 2009a). Collagen fibrils decrease the rate of demineralization when present in a larger quantity, because the presence of collagen makes difficult the acid diffusion into the tissue, thus minimizing the erosion process (KLONT, TEN CATE, 1991; KLETER et al, 1994). Dentin also contains enzymes called matrix metalloproteinases (MMPs) that are activated by a drop in pH below 4.5. However, degradation of the dentin organic matrix is only possible after neutralization of saliva pH, since, although these enzymes are activated at acidic pH, they do not have the capacity to degrade the dentin matrix at the same pH (TJADERHANE et al, 1998; STROBEL, HELLWIG, 2015).

Natural enzymatic inhibitors have been increasingly studied by researchers (CASTELLAN et al, 2010; ISAKSSON et al, 2014) because they present lower toxicity when compared with synthetic products and minimal side effects (SABINO et al 1999; YE et al, 1999). Green tea has been shown to be effective in reducing dentin wear under erosive and/or

abrasive conditions (KATO et al, 2009). The green tea extract, epigallocatechin 3-gallate (EGCg), was added to a gel of topical application, which showed to be able to reduce the wear in dentin significantly, compared to fluoride (KATO et al, 2010). In addition, because it is an inhibitor of MMPs, this extract led to significantly lower concentrations of hydroxyproline compared to other treatments, proving its preventive effect on dentin erosion (KATO et al, 2012).

In research on dental caries and periodontal disease, some benefits related to polyphenols from Cranberry juice (Vaccinium macrocarpon) or extracted from the fruit itself have been scientifically proven. EGCg and Proanthocyanidins have become targets of studies, since they have the capacity to reduce the inflammatory response, as well as the production and activity of proteolytic enzymes (MMPs) that favor the destruction of the extracellular matrix in periodontal disease (LA, HOWELL, GRENIER, 2009; BONIFAIT, GRENIER, 2010). Several studies have demonstrated beneficial properties for the treatment and prevention of caries and periodontal disease (FEGHALI et al, 2012; GAZZANI, DAGLIA, PAPETTI, 2012), which would probably be related to the inhibition of the production and activity of matrix metalloproteinases (LA, HOWELL, GRENIER, 2009; DÉZIEL et al, 2010; EPASINGHE et al, 2013). Based on the ability to inhibit MMPs, the possibility of this effect for the dentin tissue, aiming the maintenance of the collagen matrix, since this is of paramount importance to minimize the erosion process.

Proanthocyanidin (PA), which has a flavan 3 - ol subunit attached to C4 - C6 (C8), is a natural compound derived from fruits, vegetables, nuts, among others (HAN et al, 2003; KENNEDY, TAYLOR, 2003). Studies have shown that the PA has a high affinity with proteins rich in proline, such as collagen, besides being responsible for increasing the ability of cells to synthesize collagen (HAN et al, 2003).

Other studies have demonstrated a significant improvement in adhesion and mechanical properties at the resin-dentin attachment interface, making adhesive restorations resistant and durable (CASTELLAN et al, 2010; CASTELLAN et al, 2013).

Proanthocyanidin also has showed its effectiveness in preventing dentin erosion because it was able to reduce the dentin wear and DOM degradation when used as gel. (BOTEON et al, 2017) Other studies show that proanthocyanidin-based products, such as gels and dentifrices, may be useful in minimizing the wear of dentin exposed to erosive agents (BUENO et al, 2016; HONÓRIO et al. 2016). Therefore, proanthocyanidin-based home use

products could be extremely useful in preventing large dentin losses by patients who regularly consume acidic beverages. Other extracts have been tested as mouthrinse and have shown good results in reducing dentin wear (MAGALHÃES et al, 2009b). However, mouthrinse based on this specific agent (proanthocyanidin) have never been tested for this purpose.

On the other hand, it is known that fruits rich in proanthocyanidin such as grape and Cranberry have low pH values (Food and Drug Administration FDA – website) and although they have agents that can minimize the degradation of dentin collagen exposed to erosive agents, their low pH values may in turn accelerate this process. Thus, the literature does not clarify whether extracts of proanthocynidin at its naturally acidic pH will inhibit or induce degradation of the demineralized organic matrix.

Therefore, the aim of this study was to evaluate the efficacy of a proanthocyanidin based naturally acidic mouthrinse and a pH neutral proanthocyanidin based mouthrinse on dentin submitted to erosive challeng

# 2 DISCUSSION

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The *in situ* study was chosen because it allows reproducing similar conditions of the human oral cavity, contributing to the control of the biases inherent to this type of study and the highly controlled laboratory situation (ZERO, 1995; HONÓRIO et al, 2008). On the other hand, it requires that the work be conducted with great discretion, as it depends on the collaboration of the volunteers (ZERO, 1995; HONÓRIO et al, 2008).

Bovine dentin was the substrate selected for this study because it has been used in several studies (RIOS et al, 2006; HONÓRIO et al, 2008; KATO et al, 2009; HONÓRIO et al, 2010). It presents similarities with human teeth as to its morphology (SCHILKE et al, 2000; FONSECA et al, 2008), physiology (SCHMALZ et al, 2001) and mechanical structure (HARA et al, 2003; WEGEHAUPT et al, 2008). Bovine dentin has already been used in research on dental erosion (KATO et al, 2009; KATO et al, 2010; KATO et al, 2011), and state that this substrate is very reliable for studies involving dentin erosion and the activity of metalloproteinases.

In this study, the agent selected to minimize the progression of dentin erosion was a purified proanthocyanidin from grape seeds. This reduction probably occurs due to inhibition of the MMPs from DOM. The effectiveness of agents has been proven through several studies (HAN et al, 2003; LA, HOWELL, GRENIER, 2009; MAGALHÃES et al, 2009; KATO et al, 2010; CASTELLAN et al, 2011; BEDRAN-RUSSO et al, 2014; KATO et al, 2014; HONÓRIO et al, 2016), with the inhibition effect on DOM collagenolytic enzymes, mainly for chlorhexidine (GENDRON et al, 1999; MAGALHÃES et al, 2009; KIM et al, 2011).

Among the different ways of incorporating active principles, this *in situ* study selected the method of application in the form of a mouthrinse because it allows greater comfort to the patient, since he can use the solution daily without the need to attend the dental office. The use of agents in toothpaste to prevent or control tooth erosion is questionable because brushing may increase damage to softened dental structure after an acid challenge (ATTIN et al, 2001; LUSSI et al, 2014).

In addition, the literature demonstrates the efficacy of rinses when used with different agents in the prevention of initial erosion of the enamel (de OLIVEIRA et al, 2015), in reducing erosion and dentin abrasion (MAGALHÃES et al, 2009b), in the control of dentin

hypersensitivity (MOLINA et al, 2016) and in the protection of deciduous teeth submitted to simulated erosion (M'BAREK et al, 2014).

Fluoride was not part of this study as it has the ability to promote the formation of a metal-rich resistant acid layer or a layer of calcium fluoride that provides temporary protection against erosive challenges. Therefore, to be efficient, this agent would require multiple applications (HUYSMANS, YOUNG, GANSS, 2014).

Chlorhexidine was chosen as a positive control of this work because it showed efficacy in the inhibition of collagen degradation by MMPs and ability to decrease dentin wear (GENDRON et al, 1999; HEBLING et al, 2005; CARRILHO et al, 2007; MAGALHÃES et al, 2009b; KATO et al, 2010; BUZALAF et al, 2012; KATO et al, 2012; BUZALAF, MAGALHÃES, WIEGAND, 2014; ZARELLA et al 2015). An in *situ study* (MAGALHÃES et al, 2009b) in which volunteers performed mouthrinse with chlorhexidine after erosive challenge *ex vivo* showed that it was able to reduce dentin erosion. The literature shows that there are promising results in the inhibition of MMPs when chlorhexidine and green tea are used in the form of a mouthrinse resulting in the reduction of dental erosion (MAGALHÃES et al, 2009b). In addition, the green tea extract used in gel form also showed ability to prevent erosion in dentin (KATO et al, 2010). In this study, the group treated with chlorhexidine and treated with neutralized proanthocyanidin had the best results in the prevention of erosion, with no statistical difference between them. Thus, the use of a neutral proanthocyanidin-based mouthwash at first appears to be more advantageous than chlorhexidine rinse, since it does not present all the side effects presented by the regular use of chlorhexidine, such as: staining of teeth, loss of taste and desquamation of oral mucosae (GUIMARÃES et al, 2006).

Based on that, natural agents have been increasingly studied in the development of oral health products (KHADDAM et al, 2014). Collagen crosslinking agents were tested and the highest statistically significant inhibition of total MMP activity was observed using grape seed extract. Although it did not present a statistically significant difference when compared to the synthetic agents, this work demonstrated that the grape seed extract obtained greater inactivation of the MMPs, proving the best action of natural agents when compared to the synthetic ones (SESEOGULLARI-DIRIHAN et al, 2016).

Among natural agents there is the PA, widely available in fruits, vegetables, nuts, seeds, flowers and barks (FINE, 2000; JOSHI, KUSZYNSKI, BAGCHI, 2001). It is

biocompatible, which presents good bioactivity with dentin and is available as a renewable resource (BEDRAN-RUSSO et al, 2007; PAVAN et al, 2011; BEDRAN-RUSSO et al, 2014).

The literature shows that some agents rich in proanthocyanidin (JOSHI, KUSZYNSKI, BAGCHI, 2001; BEDRAN-RUSSO et al, 2007; BEDRAN-RUSSO et al, 2014) may be able to inhibit the action of MMPs on DOM degradation. The maintenance of DOM is important because the presence of collagen makes it difficult to diffuse the acids into the tissue, thus softening the erosion process (KLETER, 1994).

*In vitro* [(BOTEON et al, 2017) and *in situ* (HONÓRIO et al, 2014), studies in which the pH of the PA was adjusted, showed that this agent has the ability to prevent the progression of dentin wear. This study did not present the same results by using the extract *in natura* (acid pH), but showed that if pH was ajusted the results are promising. Therefore, the results of the present study have shown that the use of proanthocyanidin-based acid extracts has a more deleterious than beneficial effect.

Although there was decrease in dentin wear, further studies should be conducted to verify whether PA has the potential to bring other benefits when used as mouthrinse and if this concentration is the best one.

# 3 CONCLUSION

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The results of this study suggest that 10% neutralized proanthocyanidin mouthrinse could be a good strategy to diminish dentin wear progression.

# REFERENCES

# REFERENCES


1. Al-zarea BK. Tooth surface loss and associated risk factors in northern Saudi Arabia. SRN Dent. 2012;2012:161565.

2. Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. J Dent. 2005;33(3):243-52.

3. Attin T, Knöfel S, Buchalla W, Tütüncü R. *In situ* evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel. Caries Res 2001; 35: 216–22.

4. Barbosa CS, Barbeiro GS, Marques VR, Baldo Vde O, Buzalaf MA, Magalhães AC. Dental manifestations in bariatric patients: review of literature. J Appl Oral Sci. 2009;17 Suppl:1-4.

5. Bartlett D. Intrinsic causes of erosion. Monogr. Oral Sci. 2006;20:119-39.

6. Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. J Biomed Mater Res B Appl Biomater. 2007;80(1):268-72.

7. Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, et al. Dentin biomodification: strategies, renewable resources and clinical applications. Dent Mater. 2014;30(1):62-76.

8. Bonifait L, Grenier D. Cranberry polyphenols: potencial benefits for dental caries and periodontal disease. J Can Dent Assoc. 2010;76:a130.

9. Boteon AP, Prakki A, Buzalaf MAR, Rios D, Honório HM. Effect of different concentrations and application times of proanthocyanidin gels on dentin erosion, Am J Dent. In press, 2017.

10. Bueno TL, Boteon AP, Cardoso F, Costa MR, Souza BK Souza, Oliveira GC, Prakki A, Buzalaf MAR, Honório HM, Comparison of diferente dentifrices in organix matrix after erosion/abrasion. In: Academy of Dental Materials (ADM) Annual Meeting, 2016, Chicago-EUA. Dent Mater, 2016.

11. Buzalaf MA, Hannas AR, Magalhães AC, Rios D, Honório HM, Delbem AC. pH cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. J Appl Oral Sci. 2010 Jul-Aug;18(4):316-34.

12. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjaderhane L, et al. In vivo preservation of the hybrid layer by chlorhexidine. Journal of dental research. 2007;86(6):529-33.

13. Carvalho TS. Erosive tooth wear in children. Monogr Oral Sci. 2014;25:262-78.

14. Castellan CS, Pereira PN, Grande RH, Bedran-Russo AK. Mechanical characterization of proanthocyanidin-dentin matrix interation. Dent Mater. 2010 Oct;26(10):968-73.

15. Castellan CS, Bedran-Russo AK, Antunes A, Pereira PN. Effect of dentin biomodification using naturally derived collagen cross-linkers: one-year bond strength study. Int J Dent. 2013;2013:918010.

16. Chehal HK, Pate DH, Cohen DM, Bhattacharyya I. Dental erosion due to excessive wine consumption. Gen Dent. 2009 Sep-Oct;57(5):519-23.

17. de Oliveira TA, Scaramucci T, Nogueira FN, Simões A, Sobral MA. Effect of mouthrinses with different active agents in the prevention of initial dental erosion. Indian J Dent Res. 2015 Sep-Oct;26(5):508-13.

18. Déziel BA, Patel K, Neto C, Gottschall-Pass K, HURTA, R.A. Proanthocyanidins from the American Cranberry (Vaccinium macrocarpon) inhibit matrix metalloproteinase-2 and matrix metalloproteinase-9 activity in human prostate cancer cells via alterations in multiple cellular signalling pathways. J Cell Biochem. 2010 Oct 15;111(3):742-54.

19. Eccles JD. Tooth surface loss from abrasion, attrition and erosion. Dent Update. 1982;9(7):373-4, 376-8, 380-1.

20. Epasinghe DJ, Yiu CK, Burrow MF, Hiraishi N, Tay FR. The inhibitory effect of proanthocyanidin on soluble and collagen-bound proteases. J Dent. 2013 Sep;41(9):832-9.

21. Feghali K, Feldman M, La VD, Santos J, Grenier D. Cranberry proanthocyanidins: Natural weapons against periodontal diseases. J Agric Food Chem. 2012 Jun 13;60(23):5728-35.

22. Fine AM. Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications. Altern Med Rev. 2000 Apr;5(2):144-51.

23. Food and drug Administration, pH values of various food. https://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/ucm122561.htm. (accessed 21.02.2017).

24. Fonseca RB, Haiter-Neto F, Carlo HL, Soares CJ, Sinhoreti MA, Puppin-Rontani RM, et al. Radiodensity and hardness of enamel and dentin of human and bovine teeth, varying bovine teeth age. Archives of oral biology. 2008;53(11):1023-9.

25. Ganss C. Definition of erosion and links to tooth wear. Monogr Oral Sci. 2006;20:9-16.

26. Gazzani G, Daglia M, Papetti A. Food components with anticaries activity. Curr Opin Biotechnol. 2012 Apr;23(2):153-9.

27. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clinical and diagnostic laboratory immunology. 1999;6(3):437-9.

28. Guimarães ARD, M.A. Peres, R.S. Vieira, R.M. Ferreira et al. Self-perception of side effects by adolescents in a chlorhexidine fluoride based preventive oral health program. Journal Applied of Oral Science. 2006:(14)291-6.

29. Han B, J, Tang BW, Nimni ME. Proanthocyanidin: a natural cross-linking reagent for stabilizing collagen matrices. J Biomed Mater Res A. 2003 Apr 1;65(1):118-24.

30. Hara AT, Lussi A, Zero DT. Biological factors. Monogr Oral Sci. 2006;20:88-99.

31. Hebling J, Pashley DH, Tjaderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. Journal of dental research. 2005;84(8):741-6.

32. Honório HM, Rios D, Santos CF, Magalhães AC, Buzalaf MA, Machado MA. Effects of erosive, cariogenic or combined erosive/cariogenic challenges on human enamel: an *in situ*/*ex vivo* study. Caries Res. 2008;42(6):454-9.

33. Honório HM, Rios D, Santos CF, Magalhães AC, Delbem AC, Buzalaf MA, Machado MA. Cross-sectional microhardness of human enamel subjected to erosive, cariogenic or combined erosive/cariogenic challenges. Caries Res. 2010;44(1):29-32.

34. Honório HM, Dokko JR, Rios D, Favero CS, Scaraficci AC, Prakki A. Preventive effect of Cranberry gel on dentin submitted to erosion. In: 43rd Annual Meeting & Exhibition of the AADR and 38th Annual Meeting of the CADR, 2014, Charlotte-EUA. J Dent Res, 2014. p. 695.

35. Honório HM, Boteon AP, Kato MT, Buzalaf MAR, Prakki A, Wang L, Rios D. Effect of cranberry and proanthocyanidin in dentin erosion prevention. In: Academy of Dental Materials (ADM) Annual Meeting, 2016, Chicago-EUA. Dent Mater, 2016.

36. Huysmans MC, Chew HP, Ellwood RP. Clinical studies of dental erosion and erosive wear. Caries Res. 2011;45 Suppl 1:60-8.

37. Huysmans MC, Young A, Ganss C. The role of fluoride in erosion therapy. Monographs in oral science. 2014;25:230-43.

38. Imfeld T. Dental erosion. Definition, classification and links. Eur J Oral Sci. 1996;104(2 ( Pt 2)):151-5.

39. Isaksson H, Birkhed D, Went LK, Alm A, Nilsson M, Koch G. Prevalence of dental erosion and association with lifestyle factors in Swedish 20-year olds. Acta Odontol Scand. 2014 Aug;72(6):448-57.

40. Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci. 2006;20:44-65.

41. Joshi SS, Kuszynski CA, Bagchi D. The cellular and molecular basis of health benefits of grape seed proanthocyanidin extract. Curr Pharm Biotechnol. 2001 Jun;2(2):187-200.

42. Kaidonis JA. Oral diagnosis and treatment planning: part 4. Non-carious tooth surface loss and assessment of risk. Br Dent J. 2012 Aug;213(4):155-61.

43. Kato MT, Magalhães AC, Rios D, Hannas AR, Attin T, Buzalaf MA KATO. Protective effect of green tea on dentin erosion and abrasion. J Appl Oral Sci. 2009 Nov-Dec;17(6):560-4.

44. Kato MT, Leite AL, Hannas AR, Buzalaf MA. Gels containing MMP inhibitors prevent dental erosion *in situ*. J Dent Res. 2010 May;89(5):468-72.

45. Kato MT, Hannas AR, Leite AL, Bolanho A, Zarella BL, Santos J, et al. Activity of matrix metalloproteinases in bovine versus human dentine. Caries research. 2011;45(5):429-34.

46. Kato MT, Leite AL, Hannas AR, Calabria MP, Magalhães AC, Pereira JC, et al. Impact of protease inhibitor on dentin matrix degradation by collagenase. J Dent Res. 2012 Dec;91(12):1119-23.

47. Kato Mt, Bolanho A, Zarella BL, Salo T, Tjaderhane L, Buzalaf MA. Sodium fluoride inhibits MMP-2 and MMP-9. J Dent Res 2014;93:74-77.

48. Kennedy JA, Taylor AW. Analyses of proanthocyanidins by high-performance gel permeation chromatography. J Cromatogr A. 2003 May 2;995(1-2):99-107.

49. Khaddam M, Salmon B, Le Denmat D, Tjaderhane L, Menashi S, Chaussain C, et al. Grape seed extracts inhibit dentin matrix degradation by MMP-3. Front Physiol. 2014;5:425.

50. Kleter GA, Damen JJ, Everts V, Niehof J, Ten Cate JM. The influence of the organic matrix on demineralization of bovine root dentin in vitro. J Dent Res. 1994 Sep;73(9):1523-9.

51. Kim DS, Kim J, Choi KK, Kim SY. The influence of chlorhexidine on the remineralization of demineralized dentine. J Dent 2011:39:855-862.

52. Klont B, ten Cate, J.M. Remineralization of bovine incisor root lesions in vitro: the role of the collagenous matrix. Caries Res. 1991;25(1):39-45.

53. La VD, Howell AB, Grenier D. Cranberry proanthocyanidins inhibit MMP production and activity. J Dent Res. 2009 Jul;88(7):627-32.

54. Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. Caries Res. 2004;38 Suppl 1:34-44.

55. Lussi A, Ganss C. Diagnosis of erosive tooth wear. Monogr Oral Sci. 2014;25:22-31.

56. Lussi A, Lussi J, Carvalho TS, Cvikl B. Toothbrushing after an erosive attack: will waiting avoid tooth wear? Eur J Oral Sci2014; 122: 353–9.

57. Magalhães AC, Wiegand A, Rios D, Honório HM, Buzalaf MA. Insights into preventive measures for dental erosion. J Appl Oral Sci. 2009a;17(2):75-86.

58. Magalhães AC, Wiegand A, Rios D, Hannas A, Attin T, Buzalaf MA. Chlorhexidine and green tea extract reduce dentin erosion and abrasion *in situ*. Journal of dentistry. 2009b;37(12):994-8.

59. Mahoney EK, Kilpatrick NM. Dental erosion: part 1. Aetiology and prevalence of dental erosion. N Z Dent J. 2003 Jun; 99 (2): 33-41.

60. M'Barek Z, Zouari Y, Moalla N, Zaier E, Ghoul-Mazgar S. Souakine mouth rinse solution protects deciduous enamel from simulated erosion in vitro. Eur J Paediatr Dent. 2014 Dec;15(4):407-11.

61. Moazzez R, Bartlett D. Instrinsec causes of erosion. Monogr Oral Sci. 2014; 25: 180-96.

62. Molina A, García-Gargallo M, Montero E, Tobías A, Sanz M, Martín C. Clinical efficacy of desensitizing mouthwashes for the control of dentin hypersensitivity and root sensitivity: a systematic review and meta-analysis. Int J Dent Hyg. 2016 Oct 20.

63. O'sullivan EA, Curzon ME, Roberts GJ, Milla PJ, Stringer MD. Gastroesophageal reflux in children and its relationship to erosion of primary and permanent teeth. Eur J Oral Sci. 1998;106(3):765-9.

64. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res. 2004;83(3):216-21.

65. Pavan S, Xie Q, Hara AT, Bedran-Russo AK. Biomimetic approach for root caries prevention using a proanthocyanidin-rich agent. Caries research. 2011;45(5):443-7.

66. Rios D, Honorio HM, Magalhaes AC, Delbem AC, Machado MA, Silva SM, et al. Effect of salivary stimulation on erosion of human and bovine enamel subjected or not to subsequent abrasion: an *in situ*/*ex vivo* study. Caries research. 2006;40(3):218-23.

67. Rios D, Magalhães AC, Honório HM, Buzalaf MA, Lauris JR, Machado MA. The prevalence of deciduos tooth wear in six-year-old children and its relationship with potencial explanatory factors. Oral Health Prev Dent. 2007;5(3):167-71.

68. Rios D, Honório HM, Magalhães AC, Silva SM, Delbem AC, Machado MA, et al. Scanning electron microscopic study of the *in situ* effect of salivary stimulation on erosion and abrasion in human and bovine enamel. Braz Oral Res. 2008 Apr-Jun;22(2):132-8.

69. Rytomaa I, Jarvinen V, Kanerva R, Heinonen OP. Bulimia and tooth erosion. Acta Odontol Scand. 1998;56(1):36-40.

70. Sabino KC, Gayer CR, Vaz LC, Santos LR, Felzenszwalb I,Coelho MG. In vitro and in vivo toxicological study of the Pterodon pubescens seed oil. Toxicol Lett. 1999;108(1):27–35.

71. Serra MC, Messias DC, Turssi CP. Control of erosive tooth wear: possibilities and rationale. Braz Oral Res. 2009;23 Suppl 1:49-55.

72. Seseogullari-Dirihan R, Apollonio F, Mazzoni A, Tjaderhane L, Pashley D, Breschi L, et al. Use of crosslinkers to inactivate dentin MMPs. Dent Mater. 2016 Mar;32(3):423-32.

73. Scheffel DL, Hebling J, Scheffel RH, Agee K, Turco G, de Souza Costa CA, et al. Inactivation of matrix-bound matrix metalloproteinases by cross-linking agents in acid-etched dentin. Oper Dent. 2014 Mar-Apr;39(2):152-8.

74. Scheutzel P. Etiology of dental erosion--intrinsic factors. Eur J Oral Sci. 1996;104(2 ( Pt 2)):178-90.

75. Schilke R, Lisson JA, Bauss O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Archives of oral biology. 2000;45(5):355-61.

76. Schmalz G, Hiller KA, Nunez LJ, Stoll J, Weis K. Permeability characteristics of bovine and human dentin under different pretreatment conditions. Journal of endodontics. 2001;27(1):23-30.

77. Sierpinska T, Orywal K, Kug J, Golebiewska M, Szmitkowski M. Enamel mineral content in patients with severe tooth wear. Int J Prosthodont. 2013 Sep-Oct;26(5):423-8.

78. Silverstone LM, Hicks MJ. The structure and ultrastructure of the carious lesion in human dentin. Gerodontics. 1985;1(4):185-93.

79. Strobel S, Hellwig E. The effects of matrix-metallo- proteinases and chlorhexidine on the adhesive bond. Swiss Dent J. 2015;125(2):134-45.

80. Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res. 1998 Aug;77(8):1622-9.

81. Uhlen MM, Tveit AB, Stenhagen KR, Mulic A. Self-induced vomiting and dental erosion--a clinical study. BMC Oral Health. 2014 Jul 29;14:92.

82. Wegehaupt F, Gries D, Wiegand A, Attin T. Is bovine dentine an appropriate substitute for human dentine in erosion/abrasion tests? J Oral Rehabil. 2008 May;35(5):390-4.

83. West NX, Joiner A. Enamel mineral loss. J Dent. 2014 Jun;42 Suppl 1:S2-11.

84. Ye X, Krohn RL, Liu W, Joshi SS, Kuszynski CA, McGinn TR, Bagchi M, Preuss HG, Stohs SJ, Bagchi D. The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells. Mol Cell Biochem 1999;196(1-2):99–108.

85. Zero DT. *In situ* caries models. Adv Dent Res. 1995;9(3):214-30.

86. Zero DT. Etiology of dental erosion-extrinsic factors. Eur J Oral Sci. 1996;104(2 (Pt 2)):162-77.